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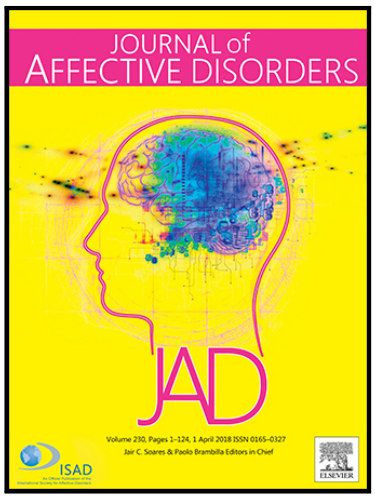

Inflammatory profiles of severe treatment-resistant depression

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Highlights

- Treatment-resistant depressed groups are rarely studied but may have high inflammation
- We assessed highly treatment-resistant inpatients before and after admission
- Findings indicate elevated inflammation in the most treatment-resistant patients
- High inflammation predicted poorer long-term outcomes after admission

Inflammatory profiles of severe treatment-resistant depression

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Abstract

Background: Treatment-resistant depression (TRD) contributes substantially to the burden of mood disorders and is undoubtedly an important subpopulation in whom there are clear unmet treatment needs. Despite a paucity of research focusing specifically on TRD, recent studies indicate that inflammatory activity may be particularly elevated in these patients.

Methods: 36 patients with TRD were investigated longitudinally before and after undertaking a specialist inpatient treatment program. 27 inflammatory proteins were compared between patients and a matched sample of non-depressed controls, as well as between treatment responders and non-responders. Treatment outcomes were calculated from depression severity scores before and after admission, and at a long-term follow-up 3-12 months after discharge.

Results: TRD patients had higher levels of numerous inflammatory proteins than controls, and elevated interleukins 6 and 8, tumour necrosis factor, c-reactive protein and macrophage inflammatory protein-1 were associated with poorer treatment outcomes. A separate set of proteins (either anti-inflammatory in nature or attenuated at baseline) showed increases during treatment, regardless of clinical response. Participants with the greatest elevations in inflammation tended to be older, more cognitively impaired and more treatment-resistant at baseline.

Limitations: The small sample and large number of comparisons examined in this study must be taken into account when interpreting these results.

Conclusions: However, this study provides empirical support for theories that more severe, chronic or treatment-resistant depressive disorders are associated with dysregulated inflammatory activity. If a predictor or predictors of response in TRD are established, improved and targeted care might be more reliably provided to this vulnerable population.

Keywords: inflammation; treatment-resistant depression; cytokines; treatment response

Introduction

Patients with treatment resistant depression (TRD) frequently do not respond to numerous treatments and present with chronic, debilitating mood disorders that are often comorbid with many physical and mental illnesses (Fekadu et al., 2009a). Despite this serious problem, evidence now suggests that even the most treatment-resistant patients can achieve sustained remission with a highly specialist, multidisciplinary, intensive and careful intervention program (Wooderson et al., 2014, 2011). Elucidating a set of factors that predicts response to treatment will be key to optimising individualised treatment choices in both bipolar and unipolar depressive disorders, and for identifying potential new targets for novel interventions (Gadad et al., 2018; Strawbridge et al., 2017). Due to the burden of TRD, improving outcomes in this population has particular scope to reduce the wider costs associated with depression (Fineberg et al., 2013). It has been suggested that TRD patients might have distinct characteristics from non-TRD patients (Kornstein and Schneider, 2001), and indirect evidence that dysregulated inflammatory activity may be a distinguishing feature (Raison et al., 2013; Strawbridge et al., 2018). Heightened inflammation might even represent a common link between treatment resistance and other core elements of TRD, such as physical illness or poor physical health (Maes et al., 2011), chronicity and/or recurrence of depressive illness (Anisman et al., 1999), cognitive impairments (Li et al., 2018) or early life trauma (Grosse et al., 2016). However, these factors are rarely assessed concurrently. Indeed, it has been uncommon for investigations of inflammation in mood disorders to assess specifically patients with TRD, naturalistic observations of treatment, long-term follow-up assessments, or comprehensive panels of inflammatory proteins. The present study explored changes of 32 inflammatory proteins occurring with naturalistic treatment for patients with severe TRD and their associations with treatment outcomes.

In response to existing evidence examining the relationship between inflammation, depression and treatment-response, the following main null hypotheses were proposed:

1) Inflammatory activity will be comparable between TRD patients and a non-depressed control group;
2) Inflammation will not be associated with clinical response to the inpatient intervention;
3) Patients' inflammatory levels will not have changed significantly between pre- and post-treatment.

Given the size and exploratory nature of this biomarker panel in this population, it was expected that our hypotheses would be true for some biomarkers only (anticipated to include the pro-inflammatory markers tumour necrosis factor (TNFα), c-reactive protein (CRP) and interleukin-6 (IL-6) based on evidence to date (Chamberlain et al., 2018; Maes et al., 1997; Strawbridge et al., 2015).

Methods

Study Design

A naturalistic investigation recruited individuals with TRD admitted to a specialist inpatient unit for people with treatment-resistant mood disorders (National Affective Disorders Unit, Bethlem Royal Hospital, South London and Maudsley NHS Foundation Trust, UK). The unit provided individualised and multi-disciplinary intervention incorporating pharmacological, psychological, occupational and physical therapies where relevant. Research assessments took place upon admission (pre-treatment) and discharge (post-treatment) from the unit. A case-control element was additionally employed to compare TRD patients with a non-depressed matched control group derived from a population-based study.

Sample Characteristics

Patients (n=36): Inpatients were eligible for the study if they met diagnostic criteria for an affective disorder (DSM-IV codes 296 or ICD-10 F30-39, assessed using the Mini International Neuropsychiatric Interview (Sheehan et al., 1998) and validated by two psychiatrists and had TRD according to the Maudsley Staging Method (MSM) multidimensional scale of treatment-resistance (Fekadu et al., 2009b),

using the cut-off score of 7.5 indicating TRD (Trevino, 2012) and a Hamilton Rating Scale for Depression 17-item (HAMD) score ≥8 (Hamilton, 1960). Treatment completion was defined as ≥4 weeks of inpatient intervention.

Controls (n=36): Non-depressed volunteers were age-, gender- and BMI-matched individuals without current psychiatric disorder, verified using the Clinical Interview Schedule-Revised (Lewis et al., 1992) and a Patient Health Questionnaire (PHQ9) score <10 (Kroenke and Spitzer, 2002). The control group were participants in a large community investigation; SELCoH study, phase-III (Hatch et al., 2011). Inflammation was assessed on one occasion for controls. Patients and controls were matched as closely as possible, to a maximum of 2 years difference in age and 5 BMI units. Blood and analysis techniques, and demographic data collection were comparable between participant samples.

This research was conducted in accordance with the Helsinki Declaration (1989) and ethical approval was granted by the Camberwell & St Giles NHS (reference 322/03) and King's College London (reference CREC/07/08-152) ethics committees. Participants provided informed written consent before providing data.

Measures

TRD patients' depression severity was examined using the HAMD at admission, discharge and a follow-up assessment 3-12 months after discharge (long-term outcome). The follow-up assessment may more accurately reflect everyday wellbeing following the treatment program, while at discharge HAMD scores could dip due to life disruption occurring in the period surrounding this (Wooderson et al., 2011). *Short-term response* was defined as HAMD score reduction of ≥50% between admission and discharge, or if criteria for remission were met (HAMD score <8). *Long-term outcome* was categorised according to depressive state at follow-up, with good outcome indicated by absent or sub-threshold depression (HAMD 0-13) and poor outcome by mild, moderate or severe depression (HAMD 14+).

At pre-treatment, the following constructs were assessed in TRD patients: Treatment-resistance within the current episode using the MSM staging tool; physical health problems using the Modified Cumulative Illness Rating Scale (MCIRS (Salvi et al., 2008)); childhood trauma history with the Childhood Trauma Questionnaire (CTQ (Bernstein et al., 1994)); cognitive function using the Mini-Mental State Examination (MMSE (Folstein et al., 1983)); number of regular pharmacological (psychotropic and non-psychotropic) treatments; medication changes (a count for any medication stopped or started during admission).

Biological Measures

Peripheral blood samples were collected by antecubital venepuncture and processed for serum extraction. After thawing, proteins were simultaneously quantified using ELISA-derived multiplex high-sensitivity assays (Meso Scale Discovery V-PLEX-Plus kit, Meso Scale Diagnostics, USA). Samples were assayed according to manufacturer's guidelines, with seven-point standard curves run in duplicate to calculate absolute concentrations and a no-template control to correct for background fluorescence. Patients and control serum samples had been stored for approximately the same time (2-4 years) and had not been thawed and refrozen before arrays were run. The two study samples were randomised across multiple assay batches; we have found high intra- ($r>0.99$) and inter-plate ($r>0.97$) correlations across proteins, indicating reliability within this sample. The coefficient of determination demonstrated high agreement between concentration and fluorescence signal ($r=0.99$). The 32 biomarkers measured were: CRP, IFNα, IFNγ, IL-10, IL-12, IL-12p70, IL-13, IL-15, IL-16, IL-17, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8 (CXCL8), TNFα, TNFβ, Eotaxin (CCL11), Eotaxin-3 (CCL26), GM-CSF, IP-10 (CXCL10), MCP1 (CCL2), MCP4 (CCL13), Mip1a (CCL3), Mip1b (CCL4), SAA, sICAM1 (sCD54), sVCAM1 (sCD106) and TARC (CCL17). These assays have been shown to provide reliable measurement of protein levels in healthy and diseased populations (Dabitao et al., 2011). Unless reported otherwise, levels are expressed as picograms per millilitre (pg/ml).

Data Analysis

Data Processing:

Protein data completeness was examined. Where >50% of datapoints were undetected they were considered uninterpretable and excluded from analyses. Where <50% fell below the assay's limit of detection, values were imputed with half the lowest level of detection (LLOD), suggested to provide <5% discrepancy with true values where 25% of values are undetected and >10% discrepancy where >50% are undetected (Croghan and Egeghy, 2003). Data distributions were examined, and biomarker data transformed using log-base10 prior to parametric test analysis. Analyses included a bootstrap of 1000 resamples.

Main comparisons:

Addressing main hypotheses, main comparisons focused on inflammatory associations with treatment outcomes, over time, and between participant groups.

1. Biomarkers were compared between TRD and control participants (hypothesis 1) using conditional logistic regressions. Where protein distributions affected the fit of regressions, they were first standardised using z scores and likelihood ratio tests assessed regressions' goodness of fit.
2. To test whether protein levels predicted response (hypothesis 2) at pre- or post-treatment, univariate logistic regressions were undertaken.
3. To assess whether inflammation changed with inpatient treatment (hypothesis 3), 2x2 mixed-factorial ANOVA analyses compared pre- and post-treatment levels (response group status was added as a between-subjects factor).

Main comparisons were subjected to a control for multiple comparisons (Simes, 1986); Simes-adjusted p-values are reported as 'adjusted'. P-values <0.05 were interpreted as statistically significant.

Exploratory comparisons:

Biomarkers were compared between dichotomous subgroups (i.e. bipolar versus unipolar, long-term good versus poor outcome, gender, physical ill health) using independent-samples t-tests. Pearson's correlations tested continuous associations with inflammatory markers (depression severity, age, BMI, number of medications and changes, childhood trauma, retrospective treatment-resistance, cognitive impairment, protein inter-correlations).

Results

TRD patient characteristics

As displayed in Table 1, patient characteristics did not differ significantly between responders and non-responders. All patients completed both pre- and post-treatment clinical assessments (n=36). However, 7 participants were unavailable for post-treatment venepuncture (post-treatment inflammatory data n=29).

20 patients (56%) were classified as treatment responders and 16 (44%) as non-responders. In the long-term, 14 patients had a good outcome (mean HAMD=9.4) and 14 had a poor outcome (mean HAMD=19.0). Patients with a poor long-term outcome were only slightly more like to have been non-responders than responders at discharge from the inpatient unit (p=0.057).

All patients were taking multiple combinations of medication on admission (see Supplementary Table 1 for a detailed summary of medications at admission and changes undergone during the inpatient program). After treatment, an increase in antipsychotic medication use could be observed, particularly in non-responder patients. The mean number of changes in psychiatric medication (number of agents started or stopped during the inpatient stay) was 3.5 for non-responders and 2.1 for responders but these did not appear related to inflammatory changes during treatment. In addition to pharmacological

therapies, many patients also undertook psychological, occupational or physical therapies. Records of these are not sufficiently complete to consider in analyses. No controls were taking NSAIDs. One responder was taking low-dose aspirin at both time points. Main comparisons were conducted with and without this patient; results were not affected so they were included to maximise statistical power.

--- Table 1 about here ---

Inflammatory marker characteristics

13/32 proteins had undetected values: IFNγ and Eotaxin-3 had <10% undetected; Mip1a, IL-5, TNFβ, IL-12p70, IL-4 and IFNα had <50% undetected. GM-CSF, IL-13, IL-1β, IL-1α and IL-2 had >50% undetected and were excluded from analyses (27 markers analysed). Non-detection was consistently numerically more frequent in control than TRD participants, suggesting lower levels in this group and this aligns with findings detailed below. Due to the high number of comparisons, Simes correction was conducted on main comparisons but due to the potential for this to have rendered putatively important findings non-significant (Streiner and Norman, 2011) we report significant effects prior to Simes (unadjusted p<0.05) in the text below.

Inflammation in TRD versus control participants

Table 2 displays baseline comparisons between TRD and non-depressed groups. Unexplained by age, gender or BMI, group differences were robust (surviving control for multiple comparisons) for 14 proteins. TRD patients had higher Eotaxin-3 (x^2(1)=29.53, p=0.001, adjusted p=0.005), IP-10 (x^2(1)=27.09, p=0.001, adjusted p=0.005), IL-8 (x^2(1)=16.61, p=0.002, adjusted p=0.007), IL-5 (x^2(1)=24.07, p=0.004, adjusted p=0.014), Mip1a (x^2(1)=29.54, p=0.001, adjusted p=0.005), Eotaxin (x^2(1)=26.54, p=0.001, adjusted p=0.005), MCP4 (x^2(1)=23.70, p=0.001, adjusted p=0.005), TARC (x^2(1)=9.29, p=0.012, adjusted p=0.027), IL-6 (x^2(1)=4.65, p=0.020, adjusted p=0.042), IL-12 (x^2(1)=3.88, p=0.025, adjusted p=0.048).

IL-16 was also higher in TRD patients, but did not survive Simes control (x^2(1)=5.31, p=0.031, adjusted p=0.054). sICAM1 (x^2(1)=23.71, p=0.001, adjusted p=0.005), sVCAM1 (x^2(1)=30.44, p=0.005, adjusted p=0.015), MCP1 (x^2(1)=5.95, p=0.010, adjusted p=0.025), and TNFβ (x^2(1)=8.36, p=0.010, adjusted p=0.025) were lower in the TRD group.

--- Table 2 about here ---

Inflammatory changes between pre- and post- treatment

Four inflammatory markers increased over time, although these effects were small and did not survive control for multiple comparisons: IL-10 (F(1,27)=4.97, p=0.037), MCP1 (F(1,27)=4.57, p=0.043), sICAM1 (F(1,27)=4.47, p=0.048) and IFNγ (F(1,27)=7.36, p=0.011). Figure 1 depicts these increases over time and in comparison with control levels. Supplementary Table 2 contains results of repeated-measures ANOVA tests.

--- Figure 1 about here ---

Inflammatory associations with treatment response

Attenuated pre-treatment IL-7 predicted subsequent non-response, although this was not significant after Simes adjustment (x^2(1)=4.64, p=0.022, adjusted p=0.616). No other inflammatory markers differed significantly between responders and non-responders (see Supplementary Table 3).

Inflammatory associations with long-term treatment outcome

Higher post-treatment pro-inflammatory markers predicted poor longer-term outcomes. Poor outcome group-status was predicted by elevated IL-6 ($t(20)=3.05$, $p=0.005$) and CRP ($t(20)=2.42$, $p=0.024$) as displayed in Figure 2, and positive correlations were identified between long-term depression severity and TNFa ($r=0.47$, $p=0.026$), IL-6 ($r=0.51$, $p=0.016$), Mip1a ($r=0.43$, $p=0.047$) and IL-8 ($r=0.58$, $p=0.005$) at post-treatment. High pre-treatment MCP4 predicted a poor long-term outcome both between groups ($t(26)=2.47$, $p=0.015$) and in correlation with long-term severity ($r=0.43$, $p=0.022$).

--- Figure 2 about here ---

Inflammatory associations with patient characteristics

The only inflammatory difference between bipolar (n=12) and unipolar (n=24) TRD was higher pre-treatment IL-4 in patients with unipolar TRD ($t(31)= -2.29$, $p=0.041$). The only inflammatory association with gender was higher pre-treatment MCP1 in male than female patients ($t(34)=2.21$, $p=0.033$). Against expectations, only IL-12p70 was correlated with childhood trauma history (post-treatment: $r=0.46$, $p=0.032$) and only two markers were associated with BMI (IL-6, pre-treatment: $r=0.35$, $p=0.039$; post-treatment: $r=0.431$, $p=0.020$ and post-treatment IL-5: $r=0.48$, $p=0.008$). Positive associations between biomarkers and physical ill health were slightly more frequent; pre-treatment IL-6 ($t(34)=2.41$, $p=0.030$), IL-15 ($t(34)=2.07$, $p=0.047$) and IL-16 ($t(34)=3.26$, $p=0.011$).

Participants with more severe depression at baseline had higher TARC ($r=0.38$, $p=0.024$) and IL-4 ($r=0.33$, $p=0.048$), while discharge CRP was positively correlated with severity ($r=0.37$, $p=0.046$). Post-treatment IL-6 ($r=0.56$, $p=0.002$) and IFNa ($r=0.42$, $p=0.025$) were higher in those who had more severe depression prior to treatment. As shown in Figure 3, severity of retrospective treatment-resistance was greater in patients with elevated pre-treatment TNFα ($r=0.37$, $p=0.029$), CRP ($r=0.35$, $p=0.039$), IL-12 ($r=0.36$, $p=0.031$) and Mip1b ($r=0.35$, $p=0.034$), and post-treatment IL-16 ($r=0.42$, $p=0.025$) and TNFβ ($r=0.43$, $p=0.019$). Additionally, IL-6 correlated positively with treatment-resistance at both pre- ($r=0.34$, $p=0.043$) and post-treatment ($r=0.37$, $p=0.047$).

--- Figure 3 about here ---

Older participants had higher inflammation, identified by correlations with CRP at pre-treatment ($r=0.40$, $p=0.015$) and post-treatment ($r=0.25$, $p=0.046$), pre-treatment only with IL-6 ($r=0.42$, $p=0.012$), IL-15 ($r=0.39$, $p=0.018$), IL-16 ($r=0.52$, $p=0.001$) and SAA ($r=0.39$, $p=0.019$) and after treatment only with MCP4 ($r=0.42$, $p=0.025$). Older patients tended to take more medications, and medication load was correlated positively with numerous pro-inflammatory markers, albeit at pre-treatment only: with IL-6 ($r=0.37$, $p=0.030$), CRP ($r=0.44$, $p=0.008$), IL-16 ($r=0.53$, $p=0.001$), MCP1 ($r=0.35$, $p=0.040$), sVCAM1 ($r=0.35$, $p=0.040$), SAA ($r=0.40$, $p=0.018$), Eotaxin-3 ($r=0.34$, $p=0.045$) and the anti-inflammatory IL-10 ($r=0.38$, $p=0.023$). Baseline Eotaxin was higher in those subsequently undergoing more medication changes during treatment ($r=0.43$, $p=0.015$).

Lastly, more cognitively impaired patients had higher IL-12 both before ($r=0.38$, $p=0.022$) and after ($r=0.45$, $p=0.014$) treatment. Additional positive associations with cognitive impairment were identified for pre-treatment IL-6 ($r=0.38$, $p=0.023$), IL-17 ($r=0.43$, $p=0.008$) and IFNγ ($r=0.51$, $p=0.001$), and for post-treatment IL-7 ($r=0.45$, $p=0.014$), IL-16 ($r=0.44$, $p=0.017$), Eotaxin ($r=0.46$, $p=0.013$) and IP-10 ($r=0.50$, $p=0.006$).

Proteins were frequently inter-correlated, with the acute-phase proteins SAA and CRP most strongly inter-related ($r=0.59$, $p<0.001$) and both strongly correlated with IL-6 and IL-16. IL-6, TNFα and IP-10 most

frequently correlated with other proteins at p<0.01. A cluster mainly comprised of chemokines was also apparent, including Eotaxin, MCP4, TARC, IL-7 and Mip1b.

Discussion

There are several routes by which inflammation might play an important role in treatment resistance and response in depression. This study demonstrates that patients with TRD have higher proteomic inflammatory activity than matched, non-depressed controls and that elevated inflammation is predictive of a more severe or resistant depressive illness both retrospectively (i.e. prior to inpatient treatment, in the current episode) and prospectively (predicting more severe depressive symptoms in the months after discharge). Nonetheless, high inflammation is also indicated in older patients with a greater medication load and cognitive impairment, as well as correlating with severity of depression at the time of measurement.

Does an inflammatory state predict short-term treatment response?

Before inpatient intervention, IL-7 is lower in patients who subsequently do not respond to treatment before adjustment for multiple comparisons. While this is a novel finding, there are previous reports of attenuated IL-7 in MDD than healthy groups, even when controlling for medication and demographics (Lehto et al., 2010), and reports of negative correlations between circulating IL-7 and depression severity (Einvik et al., 2012; Hall et al., 2016). Despite this, the observed effect is small and short-term outcome is not predicted by other inflammatory markers.

Does an inflammatory state predict other measures of a poor clinical outcome?

Stronger associations are found for inflammation predicting poor long-term outcomes (which may be a more valid and relevant parameter in TRD than short-term outcome). TRD patients with a poorer long-term outcome had higher levels of pro-inflammatory markers, supporting previous findings in MDD for common proteins IL-6 (Fornaro et al., 2011), IL-8 (Eller et al., 2009) and CRP (Uher et al., 2014). Similarly, heightened TNFa appears associated with unsuccessful antidepressant treatment with support from meta-analysis (Strawbridge et al., 2015). Few studies have examined MCP4 and Mip1a in depression and this is the first evidence to our knowledge of these biomarkers predicting treatment outcome. All these potential long-term outcome predictors are prominently involved in upregulating innate immune responses, most with chemoattractant properties, suggesting early inflammatory responses may precede more severe courses of psychiatric illness (supporting findings of other innate markers predicting response in MDD; (Cattaneo et al., 2016).

In addition to prospective comparisons, cross-sectional correlations between depression severity and biomarkers are observed, particularly at pre-treatment for TARC and IL-4, and post-treatment for CRP. This pattern suggests that both innate and adaptive immune responses correlate with the severity of TRD.

Does an inflammatory state reflect prior treatment-resistant depression?

Post-treatment IL-6 and IFNα are higher in patients who were more severely depressed before admission, which might reflect a generally more severe illness or pre-existing treatment-resistance in these patients, supported by frequent correlations between biomarkers and treatment-resistance assessed using the MSM staging tool, which incorporates duration and number of treatments undertaken within the current episode alongside severity into multidimensional quantification of TRD (Fekadu et al., 2009b). Each of these correlations were positive in direction and predominantly involve pro-inflammatory markers (IL-6, TNFα, CRP, IL-12, Mip1b, IL-16, TNFβ). Most of these proteins are higher in TRD than control groups; taken

together, these findings support the theory that TRD could represent an 'inflammatory' subgroup of depression (Raison et al., 2013; Strawbridge et al., 2018) for whom anti-inflammatory treatments may be effective (Husain et al., 2017).

Is there an inflammatory state in TRD?

We replicate previous findings of elevated inflammatory activity (Köhler et al., 2018) in a population of treatment-resistant inpatients. 15 of the 27 proteins are significantly different between TRD and control participants, and this relationship does not appear affected by age, gender or BMI. Mostly, proteins are elevated in TRD (IL-8, IL-6, IL-12, MCP4, Eotaxin, Eotaxin-3, IP-10, Mip1a, IL-5, TARC, IL-16) and these are predominantly chemokines not previously been compared between depressed and non-depressed populations, to our knowledge (e.g. TARC, Eotaxin-3) and represent new potential targets for this field. In the most extensive meta-analysis of cytokines in MDD to date, IL-12 and IL-6 were elevated as we have reported, but IL-8, Mip1a and IL-5 were not significantly altered (Köhler et al., 2018) unlike the elevations seen in this study. It is possible that these proteins are over-expressed in more chronic affective disorders and could implicate Th2 inflammation in this subpopulation.

sICAM1, sVCAM1 and MCP1 are notably lower in this sample of inpatients than controls, unexpected as these chemoattractants function as part of a pro-inflammatory response and have in a few studies been reported as elevated in depression (Dimopoulos et al., 2006; Schaefer et al., 2016). Although highly speculative, it is possible that low circulating immunoglobulin levels may suggest that they have penetrated the blood-brain-barrier into the brain (Takeshita and Ransohoff, 2012), a possibility that we were not able to examine.

The *a priori* markers of interest TNFα and CRP do not significantly differ between TRD patients and controls, in contrast with previous findings (Chamberlain et al., 2018; Köhler et al., 2018; Strawbridge et al., 2015) plausibly due to lack of matching between patients and controls in previous studies. Despite this, we find evidence of a broad inflammatory state in this group of highly treatment-resistant patients characterised by high levels of Th1 and Th2 cytokines and chemokines, alongside suppression of only a few inflammatory markers. Certainly, the bidirectionality of our findings suggests a complex relationship between TRD and immune responses, which is not exclusively 'pro-inflammatory', and may reflect a system imbalance.

Is inflammation a state or trait phenomenon in TRD?

Most cytokines do not change significantly between pre- and post- inpatient timepoints. sICAM1, MCP1, IL-10, and IFNγ increase to some degree over time; although this may appear in contrast with theories and meta-analyses (Hannestad et al., 2011; Strawbridge et al., 2015) there are a number of potential explanations. IL-10 actively downregulates pro-inflammatory responses and the increase may represent a resolution of inflammation; sICAM1 and MCP1 were attenuated in TRD patients before treatment and an increase may indicate reduced inflammatory imbalance; and IFNγ is often found to be attenuated in MDD (Köhler et al., 2018) although not in the present study.

How is inflammation in TRD influenced by other factors?

Substantial previous research has linked inflammatory markers with numerous factors such as childhood trauma, BMI and age, and lack of control for these factors has been postulated as a source of inconsistency and heterogeneity in evidence to date (Strawbridge et al., 2017). We do not find differences between treatment-responders and non-responders in any of these possible modifying variables.

As expected, patients with greater retrospective treatment-resistance tended to be older and taking more medications. However, most inflammatory associations with treatment-resistance were not those related

to medication load, age or physical illness (e.g. TNFa, IL-8, IL-12). Cognitive function is not associated with other ageing or health-related factors, but cognitive impairments are greater in the presence of high IL-17, IP-10 and IFNγ in particular. This is expected, since chronic inflammation can impair cognition through a number of neurobiological routes and is a putative link between affective illnesses and cognitive symptoms (Li et al., 2018).

Although this study does not infer causality, both inflammatory aberrations and treatment-resistance may develop progressively. A variety of studies have suggested that elevations in oxidative and nitrosative stress are associated closely with inflammation and are a key mechanism in maintaining and perpetuating future psychopathological processes in a neuroprogressive fashion through e.g. membrane damage (Maes et al., 2013). The development of these disruptions to biological processes has been attributed to various causes including genetic vulnerabilities (e.g. Czarny et al., 2016) and the experience of severe and/or ongoing psychosocial stress (Miller et al., 2009), although there are likely interactive predisposing and environmental effects.

Limitations and Strengths

The exploratory nature of this study carries a number of weaknesses regarding interpretation and attribution of findings. Notably, this includes a small sample size and large number of comparisons, as well as incomplete follow-up data, non-standardisation of treatments received, the fact that TRD and control groups were recruited from different sources and the inability to account for additional factors on inflammatory activity.

The sample size and number of comparisons undertaken in this investigation render our findings vulnerable to false positive and/or false negative (masked) results. Employing Simes adjustment for multiple comparisons lessened the risk of type I error, but may have rendered potentially important findings non-significant (Streiner and Norman, 2011). We have reported unadjusted $p<0.05$ in order to maintain consideration of these tentatively significant results. In comparisons where only few markers were significant, Simes adjustment resulted in no significant results, deriving partly from the test's assumption that independent variables should be correlated. This biomarker array contains heterogeneity; not all proteins are intercorrelated, but we posit that biomarker findings cluster together suggesting an overall inflammatory pattern that is relatively coherent. Not all analyses were subject to Simes control, many of which have not yet been conducted in depression or TRD: this was considered exploratory work which has yielded promising findings.

Further limitations include the lack of ability to control for acute infection or inflammation, especially due to a known physical condition. We attempted to account for use of non-steroidal anti-inflammatory drugs, but the possibility of acute inflammatory states biasing findings is not impossible. In addition, the different recruitment sources that TRD and control groups originated from resulted in separately assayed samples across multiple batches. While the assay itself is of high quality, reliability and sensitivity (Dabitao et al., 2011), factors such as type and length of specimen storage may have induced bias by degradation of some biomarkers. Before the array was run, neither patients' or controls' serum samples had been thawed and refrozen, or were more than 4 years old. Non-detected levels were imputed using half the detection cut-off (LLOD/2), considered acceptable where levels are likely undetected due to true minimal levels present in sera but (as with all single imputation methods) this approach does succumb to reduced variance within the sample and due caution should be paid when interpreting IFNγ and Eotaxin-3 (<10% undetected), Mip1a, IL-5, TNFβ, IL-12p70, IL-4 and IFNα (10-50% undetected). Notwithstanding the promising results identified, future studies should consider these issues throughout the research process.

The wider array of markers measured is an advantage to this study, permitting a more complete picture of inflammatory activity. Consequently, our results indicate more promising biomarkers than are often

discussed, which may be important in understanding the pathophysiology of TRD as well as other mood disorders, and for using biomarkers to improve clinical care. The large number of potential modifying factors measured also enables a more complete understanding of how inflammation is altered in TRD: health- and aging-related factors appear most important for controlling in future. Despite being naturalistically recruited, our inpatient population may not be representative of the wider population of people with TRD, most of whom do not receive such specialist care. However, additional strengths of this study include the use of a population-based control group, meaning that our results are less likely to be subject to selection bias among controls and in patients, assessment of longer-term outcomes which may provide more reliable or valid indicators of treatment success (Wooderson et al., 2014). For pragmatic reasons, follow-up was not at a standardised time point and did not contain biological measurement but depression at follow-up is found to be more clearly predicted by inflammation than at treatment endpoint.

Recommendations and clinical implications

This examination was not able to take a multivariable approach to predicting treatment-response, which will likely provide more valid and replicable predictions through integrating diverse information (e.g. inflammatory and clinical variables) rather than considering singular factors (Lee et al., 2018).

We recommend assessing response at a time after treatment, to evaluate symptoms after any transient 'treatment-end' effects have subsided, and/or measuring response at multiple time points to measure continuity of wellbeing; these might represent more valid outcome examination, particularly given the tendency of TRD to fluctuate in severity over time (Vergunst et al., 2013).

Our results support existing theories of inflammation dysregulation in depression, but illustrate that factors such as age and medication must be investigated in order to obtain meaningful results. This may be particularly important in TRD studies due to associations with reduced physical health and increased medication intake. It would be useful for future work to directly compare inflammation in TRD and non-TRD participants, as well as comparisons between unipolar and bipolar mood disorders, accounting for potentially confounding factors. If an inflammatory subtype can be determined and confirmed in future work, this has considerable implications for targeting and optimising interventions.

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Author Statement

Contributors

RS was involved in the study's conception, data collection, analysis, interpretation and writing of the manuscript. JH was involved with statistical analysis and interpretation of data. TP and GB contributed to data analysis. MH and SH were involved with study conception and design. AJC was involved with

overseeing conception and design as well as data analysis and interpretation. All authors contributed to drafting/revising the article and approved the final version for publication.

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Declaration of Interest

AJC has received honoraria for speaking from Astra Zeneca and Lundbeck, honoraria for consulting with Allergan, Janssen, Livanova, Lundbeck and Sandoz and support for conference attendance from Janssen and research grant support from Lundbeck, in the last 3 years. GB has received consultancy fees and funding from Eli Lilly. MH is principal investigator of the RADAR-CNS consortium, a public private precompetitive consortium co-funded by European Commission and members of European Federation of Pharmaceutical Industries and Associations (EFPIA) including Janssen, Lundbeck, Merck, UCB and Biogen. The authors report no further competing interests in this work.

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Table 1 TRD and non-depressed control participant characteristics

	Controls n=36	*TRD n=36*	*Responders n=20*	*Non-responders n=16*	*p-value **
Gender *n female (%)*	21 (58%)	21 (58%)	11 (55%)	10 (63%)	
Age *Mean age (range)*	54.5 (28-80)	53.8 (26-82)	55.8 (40-78)	51.1 (26-82)	
BMI *Mean BMI (range)*	28.2 (20-42)	29.1 (18-46)	29.4 (21-46)	28.6 (18-44)	
Regular medications *Mean number (range)*		5.6 (3-10)[a]	5.6 (3-10)	5.5 (3-10)	
Medication changes ** *Mean number (range)*		4.2 (1-9)[b]	3.9 (1-6)	4.6 (1-9)	
Baseline depression severity *Mean HAM-D score (range)*		21.8 (10-32)	20.5 (10-32)	22.9 (12-32)	
Discharge depression severity *Mean HAM-D score (range)*		12.2 (0-22)	7.5 (0-16)	16.3 (10-22)	<0.001
Long-term depression severity *Mean HAM-D score (range)*		14.2 (5-29)[c]	10.7 (5-19)	17.27 (10-29)	
Long-term good/poor outcome *n good outcome (%)*		14 (50%)[c]	10 (71%)	4 (29%)	0.028
Treatment-resistance *Mean MSM score (range)*		11.8 (8-15)	11.4 (8-14)	12.1 (8-15)	
Age of onset (mood disorder) *Mean age (range)*		33.1 (9-61)	38.7 (11-61)	28 (9-49)	
Lifetime psychosis *n psychosis (%)*		20 (55%)	11 (55%)	9 (56%)	
Childhood trauma severity *Mean CTQ score (range)*		57.1 (34-123)[d]	50.7 (36-77)	66.1 (34-123)	
Physical illness *n illness(%)*		17 (47%)	10 (50%)	7 (44%)	
Cognitive performance *Mean MMSE score (range)*		27.4 (18-30)	26.5 (18-30)	28.1 (20-30)	

Measurements reported from baseline assessment unless otherwise stated.

* p-value, denoting difference between groups (either TRD versus control, or responder versus non-responder; the only significant differences in characteristics were between responders and non-responders). Differences are non-significant unless stated.

** number of changes in medication with inpatient treatment (i.e. starting or stopping any medication adds 1 to the count).

[a] n=35 (20 responders, 15 non-responders)

[b] n=32 (17 responders, 15 non-responders)

[c] n=28 (14 responders, 14 non-responders)

[d] n=27 (16 responders, 11 non-responders).

Table 2: Baseline inflammation in TRD and control groups

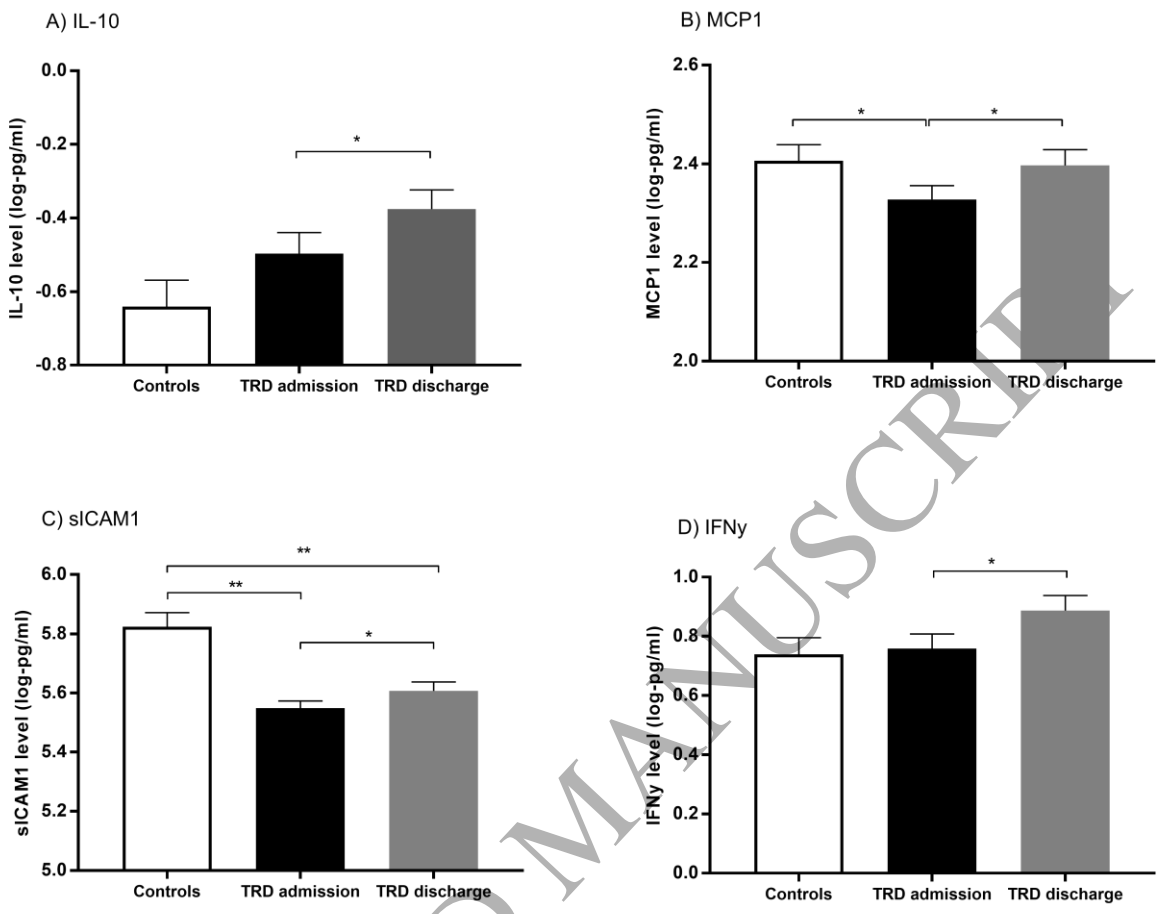
Protein	*Control group*		*TRD group*		*OR*	*95% CI*		X^2	*p*	*adj. p*
	lg-mean	*SD*	*lg-mean*	*SD*		*Lower*	*Upper*			
TNFα	0.376	0.163	0.370	0.188	0.965	0.596	1.562	0.021	0.896	0.931
IL-6	-0.140	0.315	0.016	0.400	1.811	0.996	3.293	4.649	**0.020**	**0.042**
CRP	6.570	0.568	6.415	0.697	0.737	0.433	1.254	1.322	0.572	0.672
IL-10	-0.641	0.408	-0.498	0.300	1.502	0.878	2.570	2.676	0.271	0.410
IL-8	0.935	0.176	1.348	0.592	5.858	1.551	22.133	16.609	**0.002**	**0.007**
IL-12	2.001	0.222	2.110	0.249	1.646	0.968	2.800	3.880	**0.025**	**0.048**
IL-7	1.165	0.222	1.142	0.183	0.907	0.584	1.408	0.192	0.681	0.766
IL-15	0.319	0.093	0.341	0.135	1.154	0.766	1.739	0.487	0.492	0.633
IL-16	2.228	0.168	2.352	0.219	1.688	1.025	2.781	5.310	**0.031**	0.056
IL-17	0.157	0.402	0.157	0.348	1.000	0.617	1.620	0.000	0.998	0.998
MCP1	2.406	0.188	2.313	0.159	0.506	0.277	0.927	5.950	**0.010**	**0.025**
MCP4*	1.800	0.222	2.135	0.252	5.673	1.975	16.29	23.70	**0.001**	**0.025**
Mip1b	2.083	0.153	2.129	0.298	1.250	0.752	2.075	0.770	0.405	0.576
Eotaxin*	1.969	0.179	2.252	0.166	5.410	2.038	14.363	26.54	**0.001**	**0.005**
sICAM1	5.824	0.267	5.577	0.134	0.132	0.034	0.507	23.701	**0.001**	**0.005**
sVCAM1	5.905	0.289	5.602	0.128	0.045	0.005	0.422	30.433	**0.005**	**0.015**
SAA	6.826	0.516	6.671	0.719	0.656	0.346	1.242	1.826	0.173	0.292
TARC	2.282	0.237	2.464	0.336	2.636	1.258	5.521	9.286	**0.012**	**0.027**
IP-10*	2.200	0.166	2.533	0.206	5.407	1.990	14.690	27.09	**0.001**	**0.005**
IFNγ	0.740	0.310	0.752	0.265	1.181	0.704	1.981	0.429	0.523	0.642
Eotaxin3*	0.315	0.491	1.252	0.279	5.791	2.040	16.440	29.53	**0.001**	**0.005**
Mip1a*	0.413	0.447	1.338	0.439	5.837	2.003	17.010	29.54	**0.001**	**0.005**
IL-5	-1.110	0.627	-0.275	0.532	5.385	1.810	16.025	24.065	**0.004**	**0.014**
TNFβ	-0.990	0.439	-1.271	0.392	0.449	0.240	0.842	8.358	**0.010**	**0.025**
IL-12p70	-1.214	0.524	-1.290	0.476	0.808	0.464	1.405	0.586	0.449	0.606
IL-4	-2.025	0.371	-1.964	0.762	1.104	0.699	1.743	0.182	0.730	0.788
IFNα*	-0.308	0.472	-0.429	0.262	0.704	0.425	1.166	3.058	0.273	0.410

Bold text indicates significant effects.

* due to separation between patient and control data distributions, a conditional logistic regression with firth penalisation was undertaken

lg-mean=log-transformed mean value, SD=standard deviation, OR=odds ratio (exponent of beta coefficient), 95% CI=95% confidence intervals, p = unadjusted p value before Simes control for multiple comparisons, adj. p= p value after Simes control for multiple comparisons.

Figure 1: Inflammatory marker increases during inpatient treatment



Comparison of protein levels between controls (white) and TRD patients at pre-treatment (black) and post-treatment (grey) for (A) IL-10, (B) MCP1, (C) sICAM1 and (D) IFNy. Bars denote log transformed mean values and error bars as standard error of the mean. N.B. axes do often not begin at 0 to express group differences clearly for each protein.
* = significant differences at $p < 0.05$ (unadjusted); ** = significant differences at $p < 0.01$ (unadjusted).

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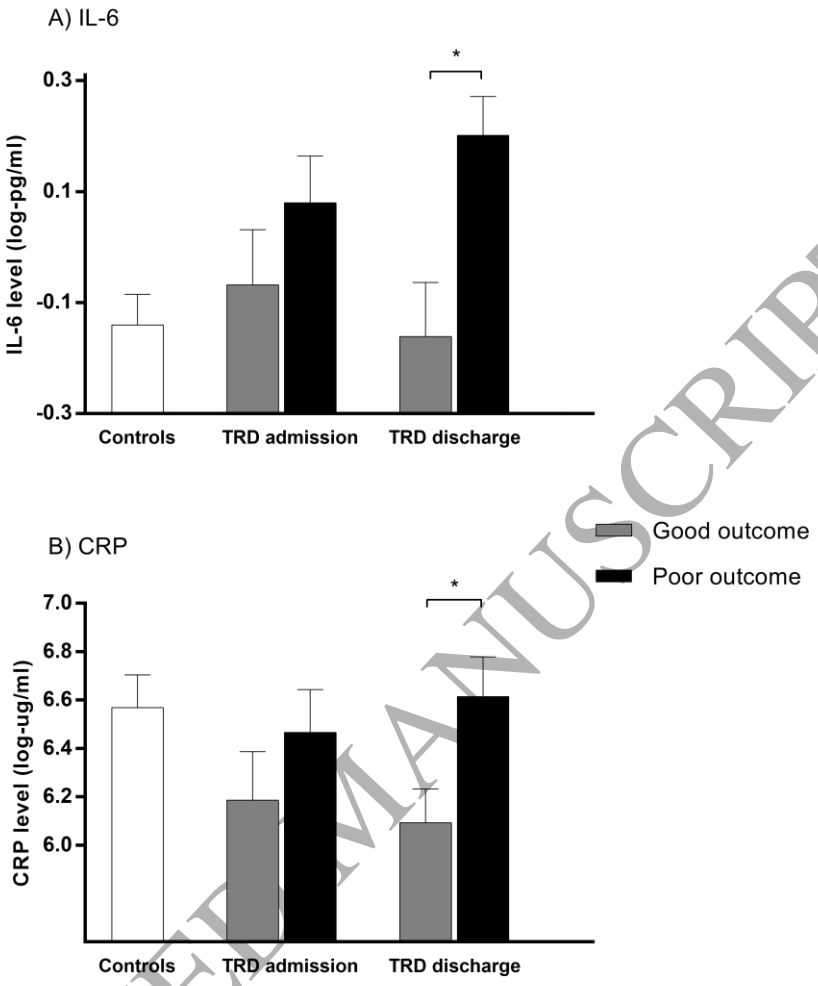
Figure 2: Inflammatory markers associated with subsequent poor long-term outcomes



Comparison of post-treatment protein levels and poor outcome in the year following discharge from the inpatient service for (A) IL-6 and (B) CRP. Displayed are biomarker levels for patients with a good outcome (grey) and poor outcome (black) at post-treatment as well as pre-treatment and control levels (white). Bars denote log-transformed mean values and error bars as standard error of the mean. N.B. axes do often not begin at 0 to express group differences clearly for each protein.
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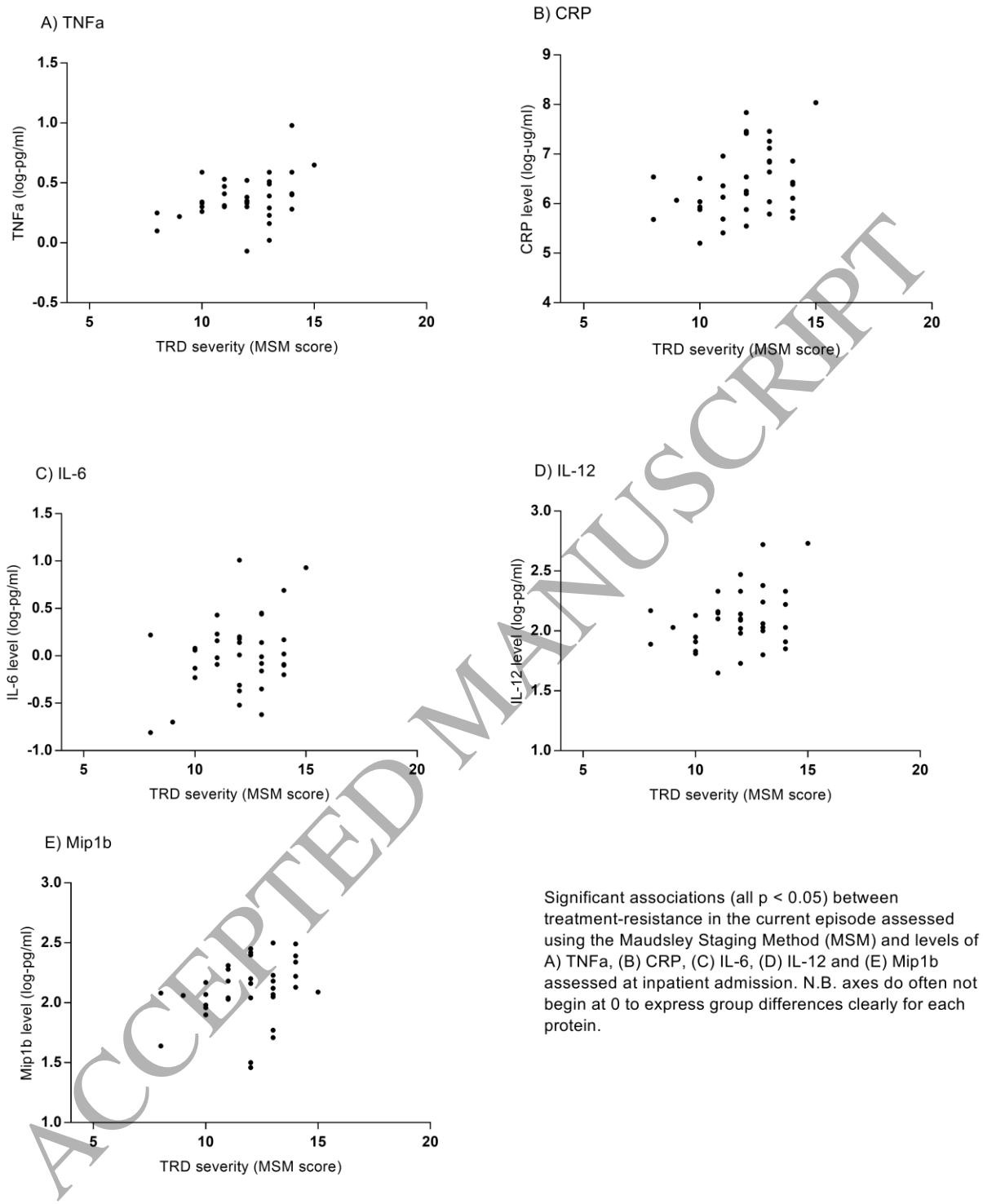
Figure 3: Baseline cytokine correlations with retrospective severity of treatment resistance



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Significant associations (all p < 0.05) between treatment-resistance in the current episode assessed using the Maudsley Staging Method (MSM) and levels of A) TNFa, (B) CRP, (C) IL-6, (D) IL-12 and (E) Mip1b assessed at inpatient admission. N.B. axes do often not begin at 0 to express group differences clearly for each protein.